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<p>(54) Title: PREVENTION OF OVARIAN CANCER BY ADMINISTRATION OF A VITAMIN D COMPOUND (57) Abstract The present invention relates to methods for preventing the development of epithelial ovarian cancer by administering a Vitamin D compound in an amount capable of increasing apoptosis in non-neoplastic ovarian epithelial cells of the female subject.</p> <p>BEST AVAILABLE COPY</p>		

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**PREVENTION OF OVARIAN CANCER
BY ADMINISTRATION OF A VITAMIN D COMPOUND**

FIELD OF THE INVENTION

This application is a continuation-in-part of U.S. Serial No. 08/713,834 filed September 13, 1996. The present invention relates generally to methods of preventing the development of ovarian cancer by administering Vitamin D compounds including Vitamin D, and biologically active analogues and derivatives thereof.

BACKGROUND OF THE INVENTION

Ovarian cancer is the fourth leading cause of cancer deaths among women in the United States and causes more deaths than all other gynecologic malignancies combined. In the United States, a woman's lifetime risk of developing ovarian cancer is 1 in 70. In 1992, about 21,000 cases of ovarian cancer were reported, and about 13,000 women died from the disease. Chapter 321, *Ovarian Cancer*, Harrison's Principles of Internal Medicine, 13th ed., Isselbacher et al., eds., McGraw-Hill, New York (1994), pages 1853-1858; *American Cancer Society Statistics*, Cancer J. Clinicians, 45:30 (1995). Epithelial ovarian cancer, the most common ovarian cancer, has a distinctive pattern of spread: cancer cells may migrate through the peritoneum to produce multiple metastatic nodules in the visceral and parietal peritoneum and the hemidiaphragms. In addition cancer cells metastasize through the lymphatic and blood vessels to areas such as the liver, lung and brain. Early stage ovarian cancer is often asymptomatic and is detected coincidentally by palpating an ovarian mass on pelvic examination. In premenopausal patients, about 95% of these masses are benign. Even after menopause, 70% of masses are benign but detection of any enlargement requires exploratory surgery. In postmenopausal women with a pelvic mass, a markedly elevated serum CA-

125 level of greater than 65 U/ml indicates malignancy with a 96% positive predictive value. Chapter 321, *Ovarian Cancer*, Harrison's Principles of Internal Medicine, *supra*.

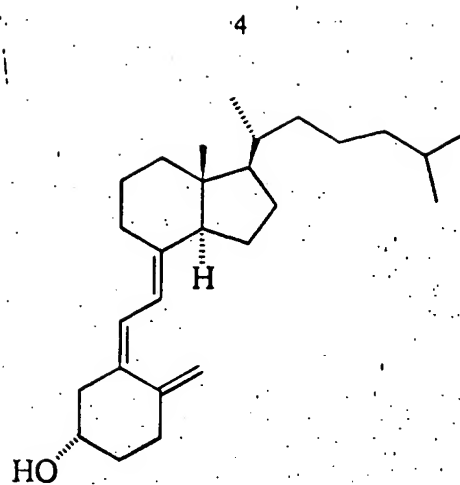
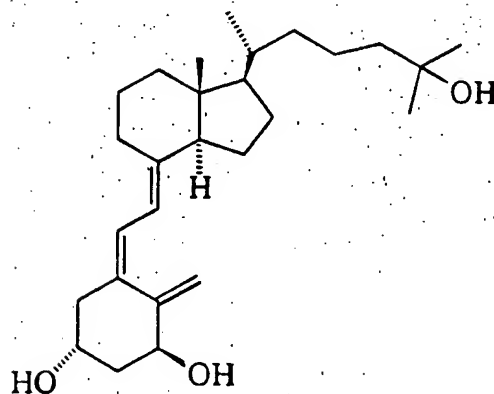
Epithelial ovarian cancer is seldom encountered in women less than 35 years of age. Its incidence increases sharply with advancing age and peaks at ages 75 to 80, with the median age being 60 years. The single most important risk factor for this cancer is a strong family history of breast or ovarian cancer. In families in which ovarian, breast, endometrial or colon cancer can be tracked as an apparent autosomal dominant trait, the risk of this cancer can be as high as 50%. Having a single first-degree relative with ovarian cancer increases a woman's risk by at least three-fold, and having a personal history of breast or colorectal cancer increases the risk of subsequently developing ovarian cancer by two-fold. Chapter 321, *Ovarian Cancer*, Harrison's Principles of Internal Medicine, *supra*. In addition, those with identifiable genetic mutations in genes such as BRCA1 also have an increased risk. Baker et al., *Etiology, Biology, and Epidemiology of Ovarian Cancer*, Seminars in Surgical Oncology 10: 242-248, 1994; Amus et al., *Genetic Epidemiology of Epithelial Ovarian Cancer*, Cancer 71: 566-72, 1993; Whitmore, *Characteristics Relating To Ovarian Cancer Risk: Implications for Preventing and Detection*, Gynecologic Oncology 55, 515-19, 1994. Oncogenes associated with ovarian cancers include the HER-2/*neu* (c-*erbB-2*) gene, which is overexpressed in a third of ovarian cancers, the *fms* oncogene, and abnormalities in the p53 gene, which are seen in about half of ovarian cancers. A number of environmental factors have also been associated with a higher risk of epithelial ovarian cancer, including a high fat diet and intake of lactose in subjects with relatively low tissue levels of galactose-1-phosphate uridyl transferase.

Previously, there has existed no established pharmaceutical approach to the prevention of ovarian cancer. For all women, especially those at high risk of developing this disease, the only available option has been

surgical removal of the ovaries, with all of the attendant risks and subsequent adverse health consequences due to resulting estrogen deficiency.

Of interest to the present invention is the disclosure of co-owned and copending U.S. Patent Application Serial.No. 08/713,834 filed September 13, 1996 entitled "Prevention of Ovarian Cancer by Administration of Progestin Products" the disclosure of which is hereby incorporated by reference. This application discloses a method for preventing the development of epithelial ovarian cancer by administering progestin products, either alone or in combination with other agents, such as estrogen products. Specifically, a method is described for preventing ovarian cancer comprising administering to a female subject an amount of progestin product effective to increase apoptosis in ovarian epithelial cells of the female subject. Apoptosis is one of the most important mechanisms used for the elimination of cells that have sustained DNA damage and which are thus prone to transformation into malignant neoplasms. Thus, increasing apoptosis of ovarian epithelial cells will prevent the transformation of non-neoplastic, including normal and dysplastic, cells into neoplastic cells.

Vitamin D is a fat soluble vitamin which is essential as a positive regulator of calcium homeostasis. In the skin 7-Dehydrocholesterol (pro-Vitamin D₃) is photolyzed by ultraviolet light to pre-Vitamin D₃, which spontaneously isomerizes to Vitamin D₃. Vitamin D₃ (cholecalciferol), the structure of which is set out below, is converted into an active hormone by hydroxylation reactions occurring in the liver to produce 25-hydroxyvitamin D₃ which is then converted in the kidneys to produce 1,25-dihydroxyvitamin D₃ (1,25-dihydroxycholecalciferol, calcitriol, 1,25(OH)₂D₃) which is transported via the blood to its classic target organs, namely, the intestine, kidney, and bones. Vitamin D₃ and 1,25-dihydroxy vitamin D₃ are shown below:

Vitamin D₃1,25-dihydroxyvitamin D₃

5

Vitamin D deficiency in childhood produces rickets, which is characterized by inadequate calcification of cartilage and bone. In adults, Vitamin D deficiency leads to softening and weakening of bones, known as osteomalacia. The major therapeutic uses of Vitamin D are divided into four categories: (1) prophylaxis and cure of nutritional rickets, (2) treatment of metabolic rickets and osteomalacia, particularly in the setting of chronic renal failure, (3) treatment of hypoparathyroidism, and (4) prevention and treatment of osteoporosis. Recommended daily dietary allowances of Vitamin D by the Food and

Nutrition Board of the United States National Research Council (1989) were 10 mg cholecalciferol (400 IU Vitamin D) daily for females age 11-24 and 5 mg cholecalciferol (200 IU Vitamin D) daily for females age 25 and older. Normal serum levels of 25-hydroxyvitamin D₃ are not closely regulated and it has a biological half-life of several weeks with blood levels typically ranging from 15 to 80 ng/mL. Serum levels of 1,25-dihydroxyvitamin D₃ are more closely regulated and typically range from 15-60 pg/mL. Serum 1,25-dihydroxyvitamin D₃ has a half-life of 6-8 hours. 1,25-dihydroxyvitamin D₃ partitions into cells by virtue of its lipophilicity, binds to intracellular receptors, and translocates to the nucleus where the complex controls the transcription of a number of genes, many of which relate to calcium metabolism. Corder et al., *Cancer Epidemiology, Biomarkers & Prevention* 2:467-472 (1993).

Certain compounds are known to upregulate the functional human Vitamin D receptor ("VDR"). For example, Santiso-Mere et al., *Molecular Endocrinology* Vol. 7, No. 7, pp.833-839 (1993) teach the expression of functional human vitamin D receptor (VDR) in *Saccharomyces cerevisiae*. This reference further teaches up-regulation of the VDR by 1,25-dihydroxyvitamin D₃. Davoodi et al., *J. Steroid Biochem. Molec. Biol.* 54: No. 3/4, pp. 147-153 (1995) relates to the effect of 1,25-dihydroxyvitamin D₃ on upregulation of the VDR. Davoodi et al. teach that progestins and trans-retinoic acid may also upregulate the VDR. Davoodi et al., at pp. 149-50.

Vitamin D and its analogues and derivatives are taught to have possible utility in the treatment, rather than prevention, of cancers by retarding tumor growth and in stimulating the differentiation of malignant cells to normal cells. For example, 1,25-dihydroxyvitamin D₃ possesses potent antileukemic activity by virtue of inducing the differentiation of leukemia cells to non-malignant macrophages.

Colston et al., *Endocrinology* Vol. 108, No. 3, 1083-1086 (1981) may have been the first to report antitumor effects of Vitamin D. This

study reported the presence of specific, high-affinity receptors for 1,25-dihydroxy-vitamin D₃ in malignant melanoma and that *in vitro* administration of 1,25-dihydroxy-vitamin D₃ produced a marked increase in cell doubling time. Sato et al., *Tohoku J. exp. Med.* 138:445-446 (1982) reported the utility of 1 α -Hydroxyvitamin D₃ in *in vivo* experiments relating to treatment of Sarcoma 180 and Lewis lung carcinoma implanted into mice. In these experiments the Vitamin D suppressed tumor growth or inhibited pulmonary metastases. Disman et al., *Cancer Research* 47: 21-25 (1987) disclose the utility of 1,25-dihydroxyvitamin D₃ in inhibiting the growth of human colonic cancer xenografts in mice. Dokoh et al., *Cancer Research* 44: 2103-2109 (1984) disclose the utility of 1,25-dihydroxyvitamin D₃ on cultured osteogenic sarcoma cells. The utility of 1,25-dihydroxyvitamin D₃ for inducing differentiation of leukemic cells is also known. See Mangelsdorf et al., *J. Cell Biol.* Vol. 98, 391-398 (1984).

Chida et al., *Cancer Research* 45: 5426-5430 (1985) describe the inhibition of the promotional phase of 7,12-dimethylbenz[a]anthracene-induced skin carcinogenesis in mice by 1,25-dihydroxyvitamin D₃. Oikawa et al., *Anti-Cancer Drugs* 2: 475-480 (1991) disclose the antitumor effect of 22-oxa-1 α ,25-dihydroxyvitamin D₃ on rat mammary tumors induced by 7,12-dimethylbenz[a]anthracene.

Vitamin D and its metabolic products while potentially useful in retarding tumor growth have the disadvantage that they are very potent calcemic agents that cause elevated blood calcium levels by stimulating intestinal calcium absorption and bone calcium resorption. Accordingly, there has been a desire in the art for Vitamin D analogues and derivatives having variant activities such that, for example, antileukemic activity is enhanced without concomitant enhancement of calcemic activity. Frampton et al., *Cancer Research* 43: 4443-4447 (1983) disclose the inhibition of human breast cancer cell growth *in vitro*. The vitamin D₃ metabolites 1,24,25-(OH)₃D₃ and 1,25,26-(OH)₃D₃ were identified as analogues which would be

effective in inhibiting tumor cell growth without exhibiting unacceptable bone resorption and hypercalcemia. Sporn et al., *Proc. Am. Assn. Cancer Res.* No. 34 Abstracts p. 622 (March 1993) report the utility of the vitamin D analogue 1,25-dihydroxy-16-ene-23-yne-26, 27-hexafluorocholecalciferol having greater
5 potency than 1,25-dihydroxycholecalciferol in differentiating HL-60 leukemic cells but which is less active in its hypercalcemic effects.

There also exists a large patent literature relating to the use of Vitamin D analogues for retarding tumor growth and treatment of leukemias. Partridge et al., U.S. Patent No. 4,594,340 teaches the syntheses of the Vitamin
10 D analogues 25,26-dehydro-1a,24R-dihydroxycholecalciferol and 25,26-dehydro-1a,24S-dihydroxycholecalciferol as differentiation inducing agents and anti-proliferation agents useful in treating osteoporosis, tumors and leukemia. DeLuca et al., U.S. Patent No. 4,800,198 discloses the use of
15 secosterol compounds sharing structural similarity with Vitamin D for inducing differentiation of malignant cells in methods of treatment of leukemic disorders.

Binderup et al., U.S. Patent No. 5,190,935 disclose Vitamin D analogues having antiproliferative effects on cancer cells. Calverly et al., U.S. Patent No. 5,206,229 disclose Vitamin D analogues exhibiting
20 antiinflammatory and immunomodulating effects which also exhibit strong activity in inducing differentiation and inhibiting undesirable proliferation of certain cells. DeLuca et al., U.S. Patent No. 5,246,925 disclose 1a-hydroxy-19-nor-vitamin D analogues which exhibit activity in arresting the proliferation of undifferentiated cells, including malignant cells, and in
25 inducing their differentiation. Ikekawa et al., U.S. Patent No. 5,278,155 disclose Fluorine-containing vitamin D₃ analogues which showed *in vitro* activity in inducing differentiation of human colonic cancer cells. DeLuca et al., U.S. Patent No. 5,373,004 disclose 26,28-methylene-1a,25-dihydroxyvitamin D₂ compounds having unique preferential calcemic activity.
30 Calverley et al., U.S. Patent No. 5,374,629 disclose Vitamin D analogues

having antiinflammatory and immunomodulating effects as well as strong activity in inducing differentiation and inhibiting proliferation of cancer cells. DeLuca et al., U.S. Patent No. 5,380,720 disclose 1 α -hydroxy-22-iodinated vitamin D₃ compounds capable of inducing relatively high differentiation of malignant cells. Hansen et al., U.S. Patent No. 5,387,582 disclose Vitamin D analogues having activity in inducing differentiation of cancer cells and skin cells. Posner et al., U.S. Patent No. 5,389,622 disclose a Vitamin D₃ analogue having growth inhibition activities against murine keratinocyte cells. Calverley et al., U.S. Patent No. 5,401,731 disclose Vitamin D analogues having activity in the prophylaxis of autoimmune diseases.

Neef et al., U.S. Patent No. 5,411,949 disclose 23-Oxa-derivatives of Vitamin D having proliferation inhibiting and cell-differentiation effects. Doran et al., U.S. Patent No. 5,428,029 disclose Vitamin D₃ fluorinated analogues as agents for the treatment of tumors such as breast cancer, as agents for the treatment of neoplastic diseases such as leukemia, and as agents for the treatment of sebaceous gland diseases. Neef et al., U.S. Patent No. 5,446,035 disclose 20-methyl-substituted Vitamin D derivatives exhibiting improved induction of cell differentiation as compared to calcitriol in an HL-60 cell line. Baggiolini et al., U.S. Patent No. 5,451,574 and No. 5,512,554 disclose Vitamin D₃ fluorinated analogues as agents for treatment of cancer, such as leukemia and or hyperproliferative skin diseases such as psoriasis. DeLuca et al., U.S. Patent No. 5,484,782 disclose (E)-20(22)-dehydrovitamin D compounds having relatively high HL-60 cell differentiation activity. Neef et al., U.S. Patent No. 5,532,228 disclose Vitamin D derivatives having cell proliferation-inhibiting and cell-differentiating activity. DeLuca et al., U.S. Patent No. 5,536,713 disclose 19-nor-Vitamin D₃ compounds with substituents at the 2-position which exhibit activity in inducing differentiation of malignant cells with little or no bone calcification activity. Dore et al., U.S. Patent No. 5,547,947 disclose methods of inducing inhibition or loss of cell proliferation in solid tumors utilizing a

Vitamin D₃ analogue alone or in combination with a trans retinoic acid. Grue-Sorensen et al., U.S. Patent No. 5,554,599 disclose 22-thio Vitamin D derivatives exhibiting antiinflammatory and immunomodulating effects which also exhibit strong activity in inducing differentiation and inhibiting undesirable proliferation of certain cells.

The use of 1,25-dihydroxyvitamin D₃ for treatment of gynecologic neoplasms including ovarian carcinomas is proposed in various references, but its efficacy against ovarian cancer cells is unclear. Moreover, there is no suggestion that Vitamin D will inhibit conversion of non-neoplastic ovarian cells to neoplastic ovarian cells or will promote apoptosis in non-neoplastic ovarian cells. Specifically, Christopherson et al., *Am. J. Obstet Gynecol.* Vol 155, No. 6, 1293-1296 (1986) report that 1,25-dihydroxhcholecalciferol is useful in inhibiting the replication of various malignant human cells but that administration of 1,25-dihydroxhcholecalciferol in ovarian adenocarcinoma cells was associated with an increase in the rate of cancer cell growth when treated at a concentration of 10 nmol/L. In contrast, Saunders et al., *Gynecologic Oncology* 44: 131-136 (1992); and Saunders et al., *Gynecologic Oncology* 51: 155-159 (1993) report the *in vitro* inhibition of endometrial carcinoma cell growth by the combination of 1,25-dihydroxyvitamin D₃ with the antineoplastic agent carboplatin; and Saunders et al., *AntiCancer Drugs* 6 562-569 (1995) report inhibition of growth in breast and ovarian carcinoma cells by 1,25-dihydroxyvitamin D₃, when combined with retinoic acid and dexamethasone. Thus, based on the results of these studies, it is unclear whether Vitamin D is itself useful for the inhibition of ovarian cancer cell growth. More significantly, none of these studies describe the effect, or suggest any effect, of Vitamin D on growth or apoptosis of non-neoplastic ovarian epithelial cells.

Similarly, while references suggest that Vitamin D may be effective to induce apoptosis in breast cancer cells, those references do not suggest that Vitamin D may effect the growth or apoptosis of non-neoplastic breast cells. For example, Welsh, *Biochem. Cell Biol.* 72: 537-545 (1994)

discloses the *in vitro* use of 1,25-dihydroxyvitamin D₃ in combination with the antiestrogen 4-hydroxytamoxifen to induce apoptosis in the breast cancer cell line MCF-7. However, Welsh makes no suggestion that Vitamin D₃ can induce apoptosis in normal or non-malignant cells.

5 The teachings of Narvaez et al., *Endocrinology* Vol. 137, No. 2 pp 400-409 (1996) are in accord with the references discussed above. Narvaez et al. teach (1) that Vitamin D can have effects on malignant cells, but the effects are cell type specific and unpredictable and (2) that, to the extent tested, Vitamin D did not have any effect on non-malignant cells. Specifically,
10 Narvaez et al., teach that 1,25-dihydroxyvitamin D₃ is a negative growth regulator of breast cancer epithelial cells and that its effects are mediated via the nuclear vitamin D receptor (VDR). The reference also suggests that the reduction in the *in vitro* growth of the MCF-7 breast cancer cell line in response to 1,25-dihydroxyvitamin D₃ is associated with morphological and
15 biochemical evidence of cancer cell death by apoptosis. Narvaez et al. disclose selection of a variant line of MCF-7 cells resistant to the growth inhibitory effects of 1,25-(OH)₂D₃. The MCF-7^{D3Res} cells express the VDR but are resistant to induction of apoptosis in response to 1,25-(OH)₂D₃ and structurally related compounds. Despite vitamin D₃ resistance, the MCF-
20 7^{D3Res} cells are sensitive to induction of apoptosis in response to antiestrogens.

 Narvaez et al. further teach that Vitamin D had no apoptotic effect on the normal cells which they studied. Specifically, the reference teaches that doses of the vitamin D analog EB1089 which cause breast tumor regression in rats have no growth or apoptotic effects *in vivo* on normal
25 intestine and kidney cells of rats treated with the analog. Narvaez et al. further investigated the possibility that 1,25-dihydroxyvitamin D₃ might be able to induce apoptosis in cell lines of normal tissues such as intestinal crypt cells and normal renal epithelial cells which express high levels of the VDR and known vitamin D₃-regulated proteins. Although the 1,25-dihydroxy vitamin
30 D₃ induced vitamin D dependent proteins in both cell lines, no evidence of

apoptosis was observed even when the cells were treated with 500 nM 1,25-dihydroxy vitamin D₃. In addition, no inhibitory effects on growth nor induction of apoptosis were observed in the intestine or kidney cells of rats treated with a vitamin D analogue (EB1089) in doses previously shown to cause breast tumor regression.

Narvaez et al. state that these and other data "suggest that although a functional VDR may be necessary for the growth regulatory effects of 1,25-(OH)₂D₃, its activation is not sufficient for triggering these effects. Thus, we hypothesize that induction of apoptosis by the 1,25-(OH)₂D₃-VDR complex is cell type specific." Accordingly, although the effects of Vitamin D are mediated by the VDR, the expression of the receptor by cells does not determine how they will respond to Vitamin D. For example, Vitamin D has potent effects on kidney cells and intestinal cells relating to calcium homeostasis, but does not cause apoptosis. On the other hand, Vitamin D might inhibit the growth of certain malignant cell lines or cause apoptosis of such cell lines. The only specific cell types for which Narvaez et al. were able to establish apoptosis through administration of Vitamin D were certain malignant cells. Narvaez et al. observed no apoptotic effect on any non-malignant cells studied. Accordingly, although ovarian epithelial cells express the VDR it would not have been expected by those skilled in the art that Vitamin D would have apoptotic effects on normal ovarian epithelial cells.

Also of interest to the present invention is the epidemiologic study of Lefkowitz et al., *International Journal of Epidemiology* vol 23, No. 6 pp 1133-1136 (1994) reporting that sunlight exposure may reduce the risk of ovarian cancer mortality. Using population based data regarding ovarian cancer mortality in large cities across the United States, as well as geographically based long-term sunlight data reported by the National Oceanic and Atmospheric Administration, the authors found an inverse correlation between regional sunlight exposure and ovarian cancer mortality risk. The publication refers to the antineoplastic effect of vitamin D against cancer lines

and tumors as demonstrated in *in vivo* and *in vitro* studies and suggests that this antineoplastic effect may be reducing the ovarian cancer mortality rates for the regions with more sunlight. Thus, this study teaches that Vitamin D may have an effect on malignant cells. There is no teaching or suggestion that sunlight may have any effect on non-neoplastic cells or that the protective effect of sunlight may be mediated by an effect of enhanced levels of Vitamin D on non-neoplastic ovarian epithelial cells *in vivo*.

Studzinski et al., *Cancer Research* 55:4012-4022 (1995) also discuss the potential effect of Vitamin D from sunlight on retarding neoplastic progression of various cancers. Studzinski et al. refer to evidence that Vitamin D retards growth of cancer cells *in vivo* and *in vitro*, induces differentiation of cancer cells, and induces apoptosis in cancer cells, and that these effects may prevent cancer progression. Studzinski et al. do not suggest or imply that Vitamin D may have a preventative benefit through effect on non-malignant cells.

Thus, while the art reports various therapeutic activities of Vitamin D and its analogues and derivatives in retarding tumor growth, the effect of Vitamin D on ovarian carcinoma cells is unclear. Moreover there exists no suggestion that Vitamin D has activity in causing apoptosis in non-neoplastic cells or in inhibiting the conversion of non-neoplastic cells to neoplastic cells in any manner. Accordingly, there remains a need in the art for methods and compositions which will prevent cancers such as ovarian epithelial cancer by inhibiting the conversion of normal and dysplastic ovarian epithelial cells to neoplastic cells.

SUMMARY OF THE INVENTION

The present invention provides a method for preventing the development of epithelial ovarian cancer by administering an effective amount of Vitamin D compounds including Vitamin D and biologically active analogues and derivatives thereof to a female subject.

While the inventors do not wish to be bound by any particular theory, the present invention is based on the discovery that administration of Vitamin D compounds results in an accelerated rate of apoptosis *in vitro* in non-neoplastic human ovarian epithelial cells including benign and dysplastic ovarian epithelial cells. Apoptosis is one of the most important mechanisms used for the elimination of cells that have sustained DNA damage and which are thus prone to transformation into malignant neoplasms. By augmenting the apoptosis pathway, Vitamin D compounds including Vitamin D and biologically active analogues and derivatives thereof may thus enhance the efficient removal of pre-neoplastic ovarian epithelial cells, thereby decreasing the risk of developing epithelial ovarian carcinoma.

Thus, the invention provides methods of inhibiting conversion of non-neoplastic ovarian epithelial cells to neoplastic cells comprising administering to a female subject an amount of Vitamin D or a biologically active analogue or derivative thereof effective to increase apoptosis in non-neoplastic ovarian epithelial cells of a female subject. The invention further provides methods of increasing apoptosis of non-neoplastic ovarian epithelial cells of a female subject comprising administering to a female subject an amount of Vitamin D or a biologically active analogue or derivative thereof effective to increase apoptosis in ovarian epithelial cells of the subject.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally relates to methods for preventing the development of epithelial ovarian cancer by administering Vitamin D compounds in an amount effective to increase apoptosis of ovarian epithelial cells. The invention also provides a method of increasing apoptosis in ovarian epithelial cells of a female subject comprising administering to a female subject an amount of a Vitamin D compound including Vitamin D or an analogue or derivative thereof effective to increase apoptosis in ovarian epithelial cells of the female subject.

The present invention is related to the discovery that administration of 1,25-dihydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ each induced an accelerated rate of apoptosis *in vitro* in human ovarian epithelial cells. Apoptosis is a process whereby a genetic program within the cell is activated to trigger a specific series of events within the cell eventually leading to the death and efficient disposal of the cell. Richard Lockshin, Zahra Zakeri, *The Biology of Cell Death and Its Relationship to Aging in Cellular Aging and Cell Death*, pp. 167-180, 1996. Wiley-Liss Inc., Editors: N.J. Holbrook, G. Martin, R. Lockshin, C. Miligan, L. Schwartz, *Programmed Cell Death During Development of Animals in Cellular Aging and Cell Death*, pp. 181-208, 1996. Wiley-Liss Inc. P53-Dependent Apoptosis in Tumor Progression and in Cancer Therapy, Scott W. Lowe, H. Earl Ruley in *Cellular Aging and Cell Death*, pp. 209-234, 1996. Wiley-Liss, Inc.

For cells that have sustained DNA damage, apoptosis is one of the most important mechanisms used for the elimination of these cells, the preservation of which could otherwise lead to the development of malignant neoplasms. Canman et al., *DNA Damage Responses: P-53 Induction, Cell Cycle Perturbations, and Apoptosis*, *Cold Spring Harbor Symp. Quant. Biol.*, 59:277-286 (1994). Thus, the apoptosis pathway is a virtually universal safeguard to prevent the persistence and proliferation of damaged cells that can be lethal to the organism. For normal tissues, the processes of cell proliferation and cell death are usually in a steady-state balance, and the apoptosis mechanism not only serves to prevent overgrowth of tissue, but also to eliminate those cells that are aberrant and therefore prone to resist normal growth regulatory controls.

An accelerated rate of apoptosis would facilitate the destruction and thereby removal of ovarian surface epithelial cells which have defective DNA and which have the potential to transform into malignant neoplasms. Given the importance of the apoptotic pathway for removal of abnormal cells from tissues, and thus the protection of normal tissues from neoplastic

transformation, it is possible that the induction of apoptosis by Vitamin D is one mechanism underlying the effect of exposure to sunlight in reducing the risk of ovarian cancer.

The term "Vitamin D compound" including "Vitamin D" "Vitamin D analogue" or "Vitamin D derivative" as used herein includes any compound which activates the Vitamin D Receptor, by binding or otherwise, either in its form of administration or in a form to which it is converted by processing by the human body. This definition thus includes each of Vitamins D₁, D₂, D₃, D₄ and D₅ and the various known analogues and derivatives thereof and any other agent that has Vitamin D activity or is an agonist thereof and that thereby increases the rate of apoptosis in ovarian epithelial cells. It is contemplated that not only presently available Vitamin D analogues and derivatives but also Vitamin D analogues and derivatives introduced in the future will be useful according to the present invention. Given the ability to produce the VDR recombinantly as described by Santiso-Mere et al., *supra* and models for determining VDR activation efficiency those of ordinary skill would be capable of identifying suitable Vitamin D compounds useful for practice of the present invention. Suitable analogues and derivatives are expected to include but are not limited to the following: 1 α -hydroxyvitamin D₃; 25-hydroxyvitamin D₃; 1,24,25-(OH)₃D₃; 24,25-(OH)₂D₃; 1,25,26-(OH)₃D₃; 24,25-(OH)₂D₃; 1,25-dihydroxy-16-ene-23-yne-26, 27-hexafluorocholecalciferol; 25,26-dehydro-1 α ,24R-dihydroxycholecalciferol and 25,26-dehydro-1 α ,24S-dihydroxycholecalciferol; 1 α -hydroxy-19-nor-vitamin D analogues; 26,28-methylene-1 α ,25-dihydroxyvitamin D₂ compounds; 1 α -hydroxy-22-iodinated vitamin D₃ compounds; 23-Oxa-derivatives of Vitamin D; and fluorinated Vitamin D analogues; 20-methyl-substituted Vitamin D derivatives; (E)-20(22)-Dehydrovitamin D compounds; 19-nor-Vitamin D₃ compounds with substituents at the 2-position; and 22-thio Vitamin D derivatives.

Appropriate dosages to increase the induction of apoptosis of

non-neoplastic ovarian epithelial cells may be determined by those of skill in the art depending upon the identity of the Vitamin D compound and its method of administration. For example, preferred dosages of the Vitamin D compound effective to increase apoptosis of non-neoplastic ovarian epithelial cells range from 0.0001 to 1.0 mg/kg of body weight (based upon the apoptotic potency of 1,25-dihydroxyvitamin D₃) with dosages ranging from about 0.005 to 0.75 mg/kg being more preferred and dosages of about 0.05 to 0.5 mg/kg being particularly preferred. It is hypothesized that even higher dosages of 1,25-dihydroxyvitamin D₃ may be more effective in inducing apoptosis. A Vitamin D analogue that has greater potency than 1,25-dihydroxyvitamin D₃ in inducing apoptosis and/or which does not have the deleterious side effects of 1,25-dihydroxyvitamin D₃, such as hypercalcemia, could be administered at a dosage equivalent much higher than 1.0 mg/kg of 1,25-dihydroxyvitamin D₃. While the potency and bioavailability of other Vitamin D compounds and analogues may vary, those of skill in the art can determine their apoptotic potency in relation to 1,25-dihydroxyvitamin D₃ and appropriate dosages and regimens of administration through use of *in vitro* testing methods such as disclosed in the accompanying example.

Prophylactic regimens for administration of Vitamin D compounds for normal female individuals and for those at increased risk of ovarian epithelial cancer can include daily or other periodic administration of Vitamin D compounds. It is contemplated that preferred regimens for prevention of ovarian cancer may comprise periodic administration of relatively larger dosages of Vitamin D compounds on a monthly or less than monthly basis rather than more frequent administration. The larger dosage would preferably range from a dosage equivalent to at least 400 I.U., more preferably a dosage equivalent to at least 2000 I.U., or still more preferably a dosage equivalent to 4000 I.U. According to such a regimen, a larger dosage of a Vitamin D compound might induce apoptosis in a large cohort of normal or dysplastic epithelial cells which over a period of time have become

available for apoptosis. The treatment is then repeated some time later when another cohort of epithelial cells is capable of being induced for apoptosis. It is contemplated that one mode of administration may be administering the Vitamin D compound for a brief period sufficient to produce apoptotic turnover of damaged ovarian cells, followed by repeated dosing periods at intervals, for example 1, 3, 6, or 9 months or 1, 3, 5, or 10 years, selected to provide apoptotic turnover adequate to prevent malignant transformations. The most preferable mode for administration would be one that maximizes the apoptotic turnover of ovarian epithelial cells and minimizes any side effects. The advantage of a technique of using large doses of Vitamin D on an infrequent basis is that it may minimize the adverse calcemic effects of a more frequent administration of Vitamin D compounds. The efficacy of such a technique is supported by the recognition that 1,25-dihydroxyvitamin D₃, the active metabolite of Vitamin D, has a relatively short serum half-life and that its apoptotic effect may be based on transient surges in serum levels. It is also possible that the apoptotic effect may not result entirely from the interaction of 1,25-dihydroxyvitamin D₃ (or its analogues) with the Vitamin D receptor but result from the effects of other Vitamin D compounds such as 25 hydroxyvitamin D₃ on the VDR. Furthermore, the inventors do not wish to be bound by the theory presented above for the efficacy of Vitamin D in preventing epithelial ovarian cancer. While it is believed that increased apoptosis is the responsible mechanism, it may be that other mechanisms are responsible.

In one mode of practicing this invention, it is first determined that a patient does not display any signs of ovarian cancer. The patient may in the alternative or addition be determined to be a female at high risk of developing ovarian cancer. A regimen of Vitamin D product, alone or in combination with other compounds, is then prescribed for the female patient.

As a further aspect of the invention it is contemplated that Vitamin D and analogues and derivatives thereof may be co-administered with

other agents, which promote apoptosis of non-neoplastic ovarian epithelial cells. One particularly preferred class of agents are the progestins as disclosed in co-owned and copending U.S. Patent Application Serial No. 08/713,834 filed September 13, 1996, the disclosure of which is incorporated herein by reference. According to one preferred aspect of the invention, Vitamin D compounds may be administered in combination with a progestin product in amounts which will induce apoptosis of non-neoplastic epithelial cells. It is contemplated that combinations of Vitamin D compounds and progestins will exhibit not only additive but synergistic effects in the induction of apoptosis of non-neoplastic ovarian epithelial cells. In this manner the adverse physiological effects of administering larger quantities of Vitamin D compounds and of progestin products can be minimized.

The term "progestin product" as used herein includes any drug which binds to the progestin receptor and induces a progestational effect. This definition thus includes all of the known progestins, derivatives of progesterone or testosterone that have progestin activity, and progestin agonists. It is contemplated that not only presently available progestins but also progestins introduced in the future will be useful according to the present invention. The known synthetic progestins are mainly derivatives of 17-alpha-hydroxy-progesterone or 19-nortestosterone. These progestins can be classified into three groups: the pregnane, estrane, and gonane derivatives. Progestin products may be administered at a variety of dosages including at a dose less than or equal to a dose equivalent to 10 mg daily of norethindrone, more preferably less than or equal to 1 mg daily, or less than or equal to 0.2 mg daily, and possibly as low as 0.05 mg daily of a norethindrone equivalent dose. According to a preferred aspect of the invention, a vitamin D compound and a progestin may be coadministered as a pharmaceutical composition preferably in a single unit dosage, such as a tablet, for inhibiting the conversion of non-neoplastic ovarian epithelial cells to neoplastic cells. The pharmaceutical composition comprises a Vitamin D compound and a progestin

product in amounts which are together effective to increase apoptosis in non-neoplastic ovarian epithelial cells. Preferred pharmaceutical compositions include those wherein the Vitamin D compound is present at a dosage equivalent of from 0.0001 to 1.0 mg 1,25-dihydroxyvitamin D₃/kg of body weight and wherein the progestin product is present at a dosage less than, or equal to, a dosage equivalent to 10 mg of norethindrone or 1 mg of norethindrone. More preferred compositions comprise those wherein the Vitamin D compound is present at a dosage equivalent of from 0.005 to 0.1 mg 1,25-dihydroxyvitamin D₃/kg of body weight and wherein the progestin product is present at a dosage less than or equal to a dosage equivalent to 1 mg of norethindrone.

According to another dosage regimen a progestin product may be administered at a dose higher than 10 mg daily of a norethindrone equivalent dose. The oral preparations currently on the market are: norgestrel 0.075 mg, medroxyprogesterone acetate 2.5 mg, 5.0 mg, and 10.0 mg, norethindrone 0.35 mg, and norethindrone acetate 0.50 mg but it is contemplated that any of the progestins would be useful for combination with Vitamin D.

It is hypothesized that the combination of Vitamin D and progestins would have a synergistic effect, with reduced adverse side effects, based at least in part on the ability of the progestin compounds to upregulate the VDR. For that reason, it is contemplated in another aspect of the present invention that other compounds known to upregulate the VDR may be co-administered with the Vitamin D compounds. Such compounds include Vitamin A derivatives, such as retinoic acid, and also include dexamethasone.

Another aspect of the present invention involves the use of Vitamin D in combination with hormones at levels sufficient to provide the dual benefits of contraceptive protection and prevention of ovarian cancer. As discussed above, Vitamin D can be co-administered with progestins. Similarly, Vitamin D can be co-administered with estrogens and progestins at

levels sufficient to provide contraceptive protection. The levels of estrogen and/or progestin for contraceptive protection are well known in the art. (See Speroff et al., Clinical Gynecologic Endocrinology and Infertility (Chap. 15), 4th Ed. 1989, incorporated herein by reference).

5 Yet another aspect of the present motion involves the co-administration of Vitamin D with hormones at levels sufficient for hormone replacement therapy. Estrogen is the primary agent in hormone replacement therapy. Postmenopausal women are generally given estrogen alone, or with low doses of progestins. The hormones may be administered continuously or
10 cyclically. Continuous administration is typically 0.625 mg Premarin® (a conjugated equine estrogen) daily or its equivalent, with a 2.5 mg Provera® (medroxyprogesterone acetate) daily. Cyclical administration is typically 25 consecutive days of 0.625 mg Premarin® daily, with 10 mg Provera® daily on days 16 through 25, followed by 5 days of no hormone treatment (during
15 which time these women will menstruate). (See Danforth's Obstetrics and Gynecology, Chapter 42, 7th Ed. 1994, incorporated herein by reference). Exemplary regimens according to this aspect of the present invention include doses of Vitamin D compounds with estrogen (with or without other compounds such as progestins) at levels sufficient for hormone replacement
20 therapy.

Estrogen is believed to be possibly linked to ovarian cancer. For that reason, the combination of Vitamin D with estrogen would provide a pharmaceutical composition which would reduce the risk of developing ovarian cancer.

25 "Concurrent administration" or "co-administration" as used herein includes administration of the agents together, or before or after each other. The agents may be administered by different routes. For example, one agent may be administered intravenously while the second agent is administered intramuscularly, intravenously or orally. They may be
30 administered simultaneously or sequentially, as long as they are given in a

manner sufficient to allow both agents to achieve effective concentrations in the body. The preferred manner of co-administration for all the combinations described above is a single unit dosage, such as a single tablet.

All doses given herein are appropriate for a female subject of about 60 kg weight; the dosages naturally will vary more or less depending on the weight of the subject. The doses may be increased or decreased, and the duration of treatment may be shortened or lengthened as determined by the treating physician. The frequency of dosing will depend on the pharmacokinetics parameters of the agents and the route of administration. The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered agents.

Those of ordinary skill in the art will readily optimize effective dosages and concurrent administration regimens as determined by good medical practice and the clinical condition of the individual patient. Regardless of the manner of administration, the specific dose may be calculated according to body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the ability of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein. Appropriate dosages may be ascertained through use of established assays for determining dosages in conjunction with appropriate dose-response data. The final dosage regimen will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the drug's specific activity, the

responsiveness of the subject, the age, condition, body weight, diet and sunlight exposure of the patient, the severity of any infection, time of administration and other clinical factors. Given the teachings herein those of ordinary skill would be able to determine appropriate dosage levels of Vitamin D compounds for inducing apoptosis of non-neoplastic ovarian epithelial cells.

It is contemplated that the routes of delivery of Vitamin D compounds including Vitamin D and biologically active analogues and derivatives thereof (either alone or in combination with other pharmaceuticals) could include oral, sublingual, injectable (including short-acting, depot, implant and pellet forms injected subcutaneously or intramuscularly), vaginal creams, suppositories, pessaries, rings, rectal suppositories, intrauterine devices, and transdermal forms such as patches and creams.

The term "females at high risk of developing ovarian cancer" includes females with a family history of breast or ovarian cancer, females with a prior history of breast or ovarian cancer, or females with a mutation in the BRCA1 gene or any other mutation shown to be associated with a high risk of developing ovarian cancer.

Other aspects and advantages of the present invention will be understood upon consideration of the following illustrative examples.

EXAMPLE 1

Example 1 addresses the effect of administration of Vitamin D on human ovarian epithelial cells. According to this example, a cell culture derived from normal ovarian epithelial cells was plated in 24 well plates at a concentration of 100,000 cells per well. After 24 hours, the wells were treated with 1,25-dihydroxyvitamin D₃ at a 100nM concentration or control medium, and allowed to incubate for 96 hours. All experiments were carried out in triplicate. After 96 hours, cell lysates were extracted from each of the wells, and the cytoplasmic fraction was normalized for cell number and analyzed for DNA-histone complexes indicative of apoptosis using a cell death ELISA

(Boehringer, Mannheim). A significant (300%) increase in apoptosis ($p=.01$) was measured in the human ovarian epithelial cells treated with Vitamin D as compared with the controls.

EXAMPLE 2

5 A spontaneously transformed yet non-malignant epithelial cell culture derived from normal human ovarian epithelial cells was plated in pronectin coated 6-well dishes at a concentration of 250,000 cells per well. The cells were allowed to plate and then grow to 70% confluence. The wells were then washed, and the medium was replaced with phenol red free, dextran,
10 charcoal treated medium containing 2% fetal calf serum, and treated with 500 ng/ml of $24,25 (OH)_2D_3$ for 72 hours. The cells were harvested, centrifuged, and the resultant cell pellets were resuspended in lysis buffer. DNA was precipitated using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Equal amounts of DNA were then subjected to
15 electrophoresis on a horizontal 1.5% agarose gel containing ethidium bromide and visualized under UV illumination. On electrophoresis, DNA laddering (the hallmark of apoptosis) was observed in cells treated with $24,25$ Vitamin D_3 , but not in the control, untreated cells.

While the above studies relate to non-neoplastic ovarian
20 epithelial cells, it is further hypothesized that administration of Vitamin D can prevent breast cancer by causing apoptosis of non-neoplastic breast cells. Prevention of breast cancer could be achieved by administration of Vitamin D, alone or in combination with other agents and/or VDR upregulators, in an amount sufficient to cause apoptosis of non-neoplastic breast cells.

25 Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description on the presently preferred embodiments thereof. Consequently the only limitations which should be placed upon the scope of the present invention are those that appear in the appended claims.

WHAT IS CLAIMED IS:

1. A method of inhibiting conversion of non-neoplastic ovarian epithelial cells to neoplastic cells comprising administering to a female subject an effective amount of a Vitamin D compound.
- 5 2. The method of claim 1 wherein Vitamin D compound is administered at a dosage equivalent of from 0.0001 to 1.0 mg 1,25-dihydroxyvitamin D₃/kg of body weight.
- 10 3. The method of claim 2 wherein Vitamin D compound is administered at a dosage equivalent of from 0.005 to 0.1 mg/kg 1,25-dihydroxyvitamin D₃ of body weight.
- 15 4. The method of claim 1 wherein the Vitamin D compound is 1,25-dihydroxyvitamin D₃.
5. The method of claim 1 comprising concurrent administration of a progestin product.
- 20 6. The method of claim 1 further comprising concurrent administration of a Vitamin A metabolite.
7. The method of claim 6 wherein the Vitamin A metabolite is retinoic acid.
- 25 8. The method of claim 1 further comprising concurrent administration of dexamethasone.
9. The method of claim 1 which includes first determining
30 that such female subject does not display signs of ovarian cancer.

10. The method of claim 1 wherein the female subject is at high risk of developing ovarian cancer.

11. The method of claim 1 wherein said non-neoplastic cells are dysplastic cells.

12. A method of increasing apoptosis in non-neoplastic ovarian epithelial cells of a female subject comprising administering to a female subject an amount of a Vitamin D compound in an amount effective to induce apoptosis in non-neoplastic ovarian epithelial cells of the female subject.

13. The method of claim 12 wherein Vitamin D compound is administered at a dosage equivalent of from 0.0001 to 1.0 mg 1,25-dihydroxyvitamin D₃/kg of body weight.

14. The method of claim 13 wherein Vitamin D compound is administered at a dosage equivalent of from 0.005 to 0.1 mg/kg 1,25-dihydroxyvitamin D₃ of body weight.

15. The method of claim 12 wherein the Vitamin D compound is 1,25-dihydroxyvitamin D₃.

16. The method of claim 12 comprising concurrent administration of a progestin product.

17. The method of claim 16 wherein the progestin product is administered at a dosage less than or equal to a dosage equivalent to 10.0 mg of norethindrone.

18. The method of claim 16 wherein the progestin product is administered at a dosage less than or equal to a dosage equivalent to 1.0 mg of norethindrone.

19. The method of claim 18 wherein the progestin product is administered at a dosage less than or equal to a dosage equivalent to 0.2 mg of norethindrone.

20. The method of claim 12 further comprising concurrent administration of a Vitamin A metabolite.

21. The method of claim 20 wherein the Vitamin A metabolite is retinoic acid.

22. The method of claim 12 further comprising concurrent administration of dexamethasone.

23. The method of claim 12 wherein the female subject is at high risk of developing ovarian cancer.

24. The method of claim 12 wherein said non-neoplastic cells are dysplastic cells.

25. A pharmaceutical composition for inhibiting the conversion of non-neoplastic ovarian epithelial cells to neoplastic cells comprising a Vitamin D compound and a hormone product.

26. The pharmaceutical composition of claim 25 wherein said hormone is a progestin product.

27

27. The pharmaceutical composition of claim 26 wherein the Vitamin D compound is present at a dosage equivalent of from 0.0001 to 1.0 mg 1,25-dihydroxyvitamin D₃/kg of body weight and wherein the progestin product is present at a dosage less than or equal to a dosage equivalent to 10.0 mg of norethindrone and wherein said composition is a single unit dosage.

28. The pharmaceutical composition of claim 27 wherein the Vitamin D compound is present at a dosage equivalent of from 0.005 to 0.1 mg 1,25-dihydroxyvitamin D₃/kg of body weight and wherein the progestin product is present at a dosage less than or equal to a dosage equivalent to 1.0 mg of norethindrone.

29. The pharmaceutical composition of claim 25 wherein said hormone product is effective to provide contraceptive protection and wherein said composition is a single unit dosage.

30. The pharmaceutical composition of claim 29 wherein said hormone product comprises estrogen and progestin.

31. The pharmaceutical composition of claim 29 wherein said hormone product compound comprises estrogen.

32. The pharmaceutical composition of claim 25 wherein said hormone product is effective for hormonal replacement in post-menopausal women and said composition is a single unit dosage.

33. The pharmaceutical composition of claim 32 wherein said hormone product comprises estrogen.

34. The pharmaceutical composition of claim 32 wherein said hormone product includes estrogen and progestin.

INTERNATIONAL SEARCH REPORT

Int. Jonal Application No
PCT/US 98/11737

A. CLASSIFICATION OF SUEJECT MATTER
IPC 6 A61K31/59

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SAUNDERS, D. E. ET AL: "Inhibition of breast and ovarian carcinoma cell growth by 1,25-dihydroxyvitamin D3 combined with retinoic acid or dexamethasone"</p> <p>ANTI-CANCER DRUGS (1995), 6(4), 562-9</p> <p>CODEN: ANTDEV; ISSN: 0959-4973, XP002079311</p> <p>see abstract</p> <p>see page 564, column 1, paragraph 3 - page 566, column 2, paragraph 2; figures 2,4</p> <p style="text-align: center;">--- -/--</p>	<p>1-15, 20-24, 35,42-44</p>

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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Date of the actual completion of the international search

9 October 1998

Date of mailing of the international search report

02/11/1998

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/11737

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SAUNDERS, D. E. ET AL: "Inhibition of c-myc in breast and ovarian carcinoma cells by 1,25-dihydroxyvitamin D3, retinoic acid and dexamethasone" ANTI-CANCER DRUGS (1993), 4(2), 201-8 CODEN: ANTDEV; ISSN: 0959-4973, XP002079312 see abstract; figure 3 see page 205, column 2, paragraph 1 - page 206, column 1, paragraph 2 see page 207, column 2, paragraph 2	1-15, 20-24, 35,42-44
X	RUSTIN, G. J. S. ET AL: "Trial of isotretinoin and calcitriol monitored by CA 125 in patients with ovarian cancer" BR. J. CANCER (1996), 74(9), 1479-1481 CODEN: BJCAAI; ISSN: 0007-0920, XP002079313 see page 1479, column 1, paragraph 2 see page 1480, column 2, paragraph 1-2	1-4,6,7, 9-15,20, 21,23, 24, 35-37, 41-43
X	SAUNDERS, DWIGHT E. ET AL: "Receptors for 1,25-dihydroxyvitamin D3 in gynecologic neoplasms" GYNECOL. ONCOL. (1992), 44(2), 131-6 CODEN: GYNOA3; ISSN: 0090-8258, XP002079314 see abstract; figures 3-5 see page 135, column 2, paragraph 2-3	1-4, 9-15,23, 24, 35-38, 41-43
X	SAUNDERS D E ET AL: "REPRESSION OF C-MYC EXPRESSION IN OVARIAN CARCINOMA CELLS BY 1 25 DIHYDROXYVITAMIN D-3." TWENTY-THIRD ANNUAL MEETING OF THE SOCIETY OF GYNECOLOGIC ONCOLOGISTS, MARCH 15-18, 1992. GYNECOL ONCOL 45 (1). 1992. 83-84. CODEN: GYNOA3 ISSN: 0090-8258, XP002079315 see abstract	1-4, 9-15,23, 24, 35-38, 41-43
X	CHRISTOPHERSON, WAYNE A. ET AL: "Responsiveness of human carcinoma cells of gynecologic origin to 1,25-dihydroxycholecalciferol" AM. J. OBSTET. GYNECOL. (1986), 155(6), 1293-6 CODEN: AJOGAH; ISSN: 0002-9378, XP002079316 see abstract	1-4, 9-15,23, 24, 35-38, 41-43
X	SAUNDERS D E ET AL: "NONREPRODUCTIVE HORMONES AS BIOLOGIC MODIFIERS IN OVARIAN CARCINOMAS." TWENTY-FOURTH ANNUAL MEETING OF THE SOCIETY OF GYNECOLOGIC ONCOLOGISTS, FEBRUARY 7-10, 1993. GYNECOL ONCOL 49 (1). 1993. 118. CODEN: GYNOA3 ISSN: 0090-8258, XP002079317 see abstract	1-15, 20-24, 35,42-44

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11737

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>MOORE T B ET AL: "Differentiating effects of 1,25-dihydroxycholecalciferol (D3) on LA-N-5 human neuroblastoma cells and its synergy with retinoic acid." JOURNAL OF PEDIATRIC HEMATOLOGY/ONCOLOGY, (1995 NOV) 17 (4) 311-7. JOURNAL CODE: B57. ISSN: 1077-4114., XP002079318 United States see abstract</p> <p>-----</p>	1-44

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/11737

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-24
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

In view of the large number of compounds which are defined by the general definition(s)/formulae used in claims 1,4-14,16-44 the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and / or the compounds as mentioned in the claims, and to the general idea underlying the application.

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